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Synaptic and Neuronal Properties of Heterotopic Neurons in the Methylazoxymethanol Model of Cortical Dysplasia and Epilepsy

By

Alana Rae Pentney ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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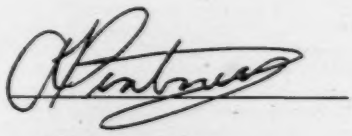

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If there's a book you really want to read,
but it hasn't been written yet,
then you must write it.

-Toni Morrison

Well behaved women rarely make history

-Laurel Thatcher Ulrich

Abstract

Neuronal migration disorders (NMDs) are commonly associated with medically-intractable partial epilepsies. Methylazoxymethanol (MAM) treatment of pregnant rats results in neuronal heterotopias in hippocampal area CA1 of the offspring. Here, we tested whether the potent anti-excitatory actions of neuropeptide Y (NPY) affected synaptic excitation of the hyperexcitable heterotopic neurons, and compared intrinsic properties of heterotopic, layer 2-3 cortical, and CA1 pyramidal neurons. NPY inhibited synaptic excitation onto CA1 cells, but was nearly ineffective on responses evoked in heterotopic cells from within the heterotopia. Heterotopic neurons differed from normal and normotopic CA1 cells in postsynaptic membrane currents, but did share many similarities with layer 2-3 cortical neurons. Thus, heterotopic cells in area CA1 appear to have been fated to join the neocortex. The lack of NPY's effects on intraheterotopic inputs may contribute to the lowered threshold for epileptiform activity in heterotopias, and may be a factor in epilepsies associated with NMDs.

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Nomenclature and Abbreviations

τ_{EPSC} – Time decay constant of the EPSC

τ_H – Time constant of current relaxation at –115 mV

AMPA - α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

APV – D-2-amino-5-phosphopentanoic acid

Ba^{2+} - Barium

CA1 – Cornus ammonis 1, area of the hippocampus

CA3 – Cornus ammonis 3, area of the hippocampus

CNS – Central nervous system

Cs^{+} - Cesium

DNA – Deoxyribonucleic acid

E15 – gestational day 15

EEG – Electro-encephalograph

EPSC – Excitatory post-synaptic current

EPSP – excitatory post-synaptic potential

GABA - γ-aminobutyric acid

I_h – Slow inwardly rectifying cation current

IR-DIC – Infrared differential interference contrast

K^{+} - Potassium

K_{IR} – Fast inwardly rectifying potassium current

MAM – Methylazoxymethanol

MAM-ac – Methylazoxymethanol-acetate

MRI – Magnetic resonance imaging

Nomenclature and Abbreviations, cont.

mRNA – Messenger ribonucleic acid

mV – Millivolts

nA – Nanoamps

NBQX – 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline

NMD – Neuronal migration disorder

NMDA – *N*-methyl-D-aspartate

NR2B – NMDA receptor subunit, type 2B

NPY – Neuropeptide Y

nS – Nanosiemens

pA - Picoamps

PNH – Periventricular nodular heterotopia

PNS – peripheral nervous system

SR –Stratum Radiatum

ZD 7288 – 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinum chloride

Chapter I: Introduction

Cortical Dysplasia in Humans

The complexity of the human brain has been described as, "a monstrous, beautiful mess. Its billions of nerve cells - called neurons - lie in a tangled web that displays cognitive powers far exceeding any of the silicon machines we have built to mimic it" (Allman, 1989). However, the placement of these neurons in the "tangled web" is vital to normal brain performance, and the convolutions of the neocortex are far better organized than their appearance suggests. Rakic (1988a) has postulated that a protocortex, or an architectonic map or plan for a future neocortex, is laid out in a gestational embryo even before the brain begins to develop. If the design of the brain is organized beforehand, even the slightest interruption or impairment in normal cortical development could lead to severe and often fatal dysgeneses with symptoms such as epilepsy, mental retardation, and dyslexia (Taylor et al., 1971; Friede, 1975; Galaburda et al., 1985; Battaglia et al., 1997) For nearly 150 years, physicians and scientists have struggled with the etiology and pathology of cortical dysplasias, and particularly with their relation to medically intractable epilepsies.

After 4 weeks of gestation, the central nervous system (CNS) of a human embryo begins to develop and the neural tube forms, proliferates, and envelops the maturing ventricular system, which eventually becomes the ventricular zone (Mischel et al., 1995). In the ventricular zone, the daughter cells of astrocytes, oligodendroglia, and neurons begin to divide and proliferate, forming the building blocks for a functional

human brain. Sets of neurons develop in the ventricular zone according to a rigid timetable, based on temporal and spatial constraints, with large neurons maturing before smaller ones. As well, all postmitotic neurons in the CNS, formed from neuroblasts, arise exclusively within the ventricular layer except in the olfactory system and cerebellum (Norman et al., 1995). After 6 weeks of gestation, and following mitosis, ventricular neurons migrate from their sites of origin along radial glial fibers to ultimately occupy a specific laminar and columnar location in the neocortex (Sidman and Rakic, 1973).

The formation of the neocortex occurs in five stages (Sidman and Rakic, 1973). Initial formation of the cortical plate can be seen between the 7^{th} and 10^{th} fetal weeks, as ventricular cells begin to migrate further outward. Over the next week, the cortical plate increases in thickness and becomes more compact. At 11-13 weeks, the cortical plate takes on a bilaminate appearance, as an inner zone populated by larger, more mature cells is visibly distinct from an outer zone composed of smaller, more densely packed neurons which have migrated more recently. During the fourth stage of neocortical formation, a secondary condensation occurs when the ventricular zone becomes progressively thinner as a large percentage of its cells migrate outward and fewer cells continue to divide, and the cortical plate again becomes homogeneous in appearance. The final stage of cortical maturation lasts from the 16^{th} week of gestation well into the postnatal period. During this time, neurons continue migrating to their final destinations in the brain, and very few neuroblasts remain in the ventricular zone (Sidman and Rakic, 1973). The first cells to arrive in the cortex

occupy what will become the deeper layers, and subsequent waves of migrating neurons travel through previously established layers in order to occupy progressively more superficial areas of the cortex (Angevine and Sidman, 1961). While ectopic factors that affect cell proliferation and differentiation may cause abnormal cortical architecture, those that hinder migration are especially dangerous during this fifth stage, as injury to radial glia can cause generalized and focal cortical dysplasia, as well as masses of displaced neurons (heterotopias) (Mischel et al., 1995). For example, this phenomenon was observed in the children of pregnant women exposed to radiation during this gestational period after the bombing of Hiroshima (Rakic, 1988b).

As early as 1859, cortical malformations caused by neuronal migration disorders (NMDs) have been associated with epilepsy in humans (Tungel, 1859). In early descriptions, heterotopias and cortical dysplasias were generally found as part of a much more complex and severe cerebral anomaly. In 1962, Layton first drew attention to the fact that heterotopias may exist as a separate anomaly, resulting in clinical symptoms, and not necessarily be part of a gross defect. A landmark study was done ten years later (Taylor et al., 1971), outlining the presence of "disordered" and "aberrant" "bizarre neurons" littered throughout cortical tissue removed from the brains of epileptic children. However, for many years the detection frequency of structural abnormalities in the brains of epileptic patients was low due to the use of inferior imaging techniques (plane x-rays, computer tomography). More recently, the development of magnetic resonance imaging (MRI) has led to a clear connection

between heterotopias and epileptogenesis (Dunn et al., 1986; Barkovich and Kjos, 1992; Battaglia et al., 1997). Of particular interest are reports of hippocampal neuronal heterotopias in epileptics (Houser, 1990; Hirabayashi et al., 1993; Raymond et al., 1994), as the hippocampus has long been implicated in the generation of temporal lobe seizure activity (Schwartzkroin, 1994).

Epilepsies in patients with NMDs are often resistant to standard anticonvulsant therapy (Palmini et al., 1991a,b, 1994), and surgical removal of dysplastic tissue has been shown to reduce seizure incidence in many of these cases (Palmini et al., 1991b; Hirabayashi et al., 1993; Raymond et al., 1995). These findings, as well as the observation that surgical outcome regarding seizure control is most strongly correlated with the amount of dysplastic area removed (Palmini et al., 1991b) suggests that disorganized cortical structures play a key role in epileptogenesis. Additionally, increased levels of the excitatory amino acid *N*-methyl-D-aspartate (NMDA) receptor subunits 2A and B, which control calcium entry through the receptor (Rafiki et al., 1998), have recently been identified in human focal cortical dysplasia (Najm et al., 2000). As calcium influx is essential for neuronal excitability and neurotransmitter release into the synaptic cleft (del Castillo-Nicolau and Stark, 1952; Katz and Miledi, 1965), high levels of NR2 subunits may increase intracellular calcium and thereby cause the release of excess glutamate, leading to hyperexcitability. However, excess intracellular calcium may lead to apoptosis as well (Orrenius et al. 1992). There are also reduced numbers of interneurons containing the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in dysplastic

regions compared with neighboring nondysplastic cortex (Ferrer et al., 1992). This combination of increased excitation and decreased inhibition in disorganized tissue strongly suggests that abnormal connectivity is a mechanism by which these lesions become foci for seizure generation.

In order to further study NMDs, several animal models of cortical dysplasias have been developed. Over 40 years ago (Falconer, 1951), the *reeler* mutant mouse was created as an autosomal genetic mutation causing neurologic disorders associated with an abnormal pattern of lamination in all cortical structures (Caviness, 1976). In other words, the neocortex of the adult *reeler* mutant mouse has been described as a reversed cortex, with the deeper layers assuming an external position (Caviness and Sidman, 1973). Additional genetic animal models of cortical malformation are the *tish* (telencephalic internal structural heterotopia) mutant rats, characterized by a distinct band of heterotopic cortex that is present bilaterally (Lee et al., 1997), and the albino *Ihara* mutant rats that harbor a subtle disarrangement of pyramidal neurons and small gaps in lamination in the hippocampus. The latter of these are particularly intriguing, as they typically develop spontaneous limbic seizures (Amano et al., 1997). By and large, these mutant models represent examples of generalized failure of differentiated neurons to properly migrate to their ultimate destinations. Although similar generalized migrational abnormalities have been observed in humans (Lo Nigro et al., 1997; Gleeson et al., 1998), most patients with NMD have more localized defects, where dysplastic areas (heterotopias) co-exist with seemingly normal tissue.

In utero insults to developing embryos tend to produce more focal cortical dysgeneses than genetic abnormalities. One such model involves the application of a freeze lesion to the neonatal rat (postnatal days 0-2) cortical plate (Dvorak and Feit, 1977; Dvorak et al., 1978), a procedure that causes a focal four-layered cortex composed of a molecular layer, two external layers, and a deep layer. This cortical malformation mimics human layered microgyria (Macbride and Temper, 1982), and the freeze lesion model has been used extensively to characterize the mechanisms of epileptogenicity associated with focal malformations. However, the microgyri created in these animals have never been shown to be the focus for abnormal seizure discharges (Chevassus-au-Louis et al. 1999b). Another animal model of cortical dysplasia involves the exposure of gestating rats to x-rays that kill dividing, postmitotic, and young migrating neurons (Altman, 1987). Depending on the timing of the X-irradiation, different types of cortical abnormalities may be modeled with this procedure (Ferrer et al., 1984). Exposure of developing embryos to methylazoxymethanol (MAM) produces similar cortical disorganization but, unlike X-irradiation, selectively affects the CNS without causing damage to other organs undergoing proliferation at the time of its administration (Balduini et al., 1991). Additionally, MAM is a more selective tool than X-irradiation in that it ablates only cells carrying out their final mitosis in the ventricular zone, and not neurons already involved in the migratory process (Cattabeni and Di Luca, 1997). For these reasons, among others, the MAM model of cortical dysplasia has become a reliable technique

by which to study neuronal migration disorders and their involvement in seizure generation.

The MAM model of cortical malformation

Cycasin, an azoxyglucoside found in the leaves and nuts of the tropical plants *Cycas circinalis* and *Cycas revoluta*, is suspected to be a causative agent of "Guam disease," the increased incidence of neurological disorders on the island of Guam, and is known to be hepatotoxic and carcinogenic in rats (Laqueur et al., 1963). Approximately 40 years ago, however, it was found that its aglycone, MAM, is the active carcinogen in the compound, and not cycasin itself (Kobayashi and Matsumoto, 1965). While studying the carcinogenicity of MAM, Spatz and Laqueur (1968) observed that a single intraperitoneal injection of the compound into pregnant rats on gestational day 15 produced a marked reduction in the size of the telencephalon in the offspring, an effect that has since been shown to be dose-dependent (Haddad et al., 1969). This finding, along with previous studies demonstrating that MAM, and its synthetic counterpart, MAM-actetate (MAM-ac), cause mutations (Smith, 1966) and methylate nucleic acids *in vitro* (Matsumoto and Higa, 1966), led to a landmark study by Nagata and Matsumoto (1969) demonstrating that MAM acts as a teratogen by methylating nucleic acids, particularly guanine, in the developing rat brain, thereby ablating dividing cells (Shank and Magee, 1967; Gillies and Price, 1993). A look at the kinetics of DNA methylation induced by MAM showed that its effects lasts from 2-24 hours after administration, and its reaction with nucleic acids is maximal at 12 hours (Matsumoto et al., 1972). With such a narrow time frame of antiproliferative activity, it is possible to use MAM to produce relatively specific damage in the brains of offspring, depending on the exact time of

drug administration during embryonic development (Chen and Hillman, 1986; Cattabeni and Di Luca, 1997).

In 1977, it was found that injection of MAM-ac into pregnant rats on gestational day 15 produces heterotopias in area CA1 of the hippocampus, which often interrupt the cell body layer (stratum pyramidale) (Singh, 1977; Chen and Hillman, 1986). The structural abnormalities of these ectopic cells were described as "reductions in the number of dendritic branches, changes in apical dendritic lengths, abnormalities in the dendritic shafts, and significant diminution in spine numbers," presumably due to inappropriate afferent stimulation or a direct toxic effect of MAM-ac on neurons (Singh, 1980). However, the mechanism by which these ectopic cells come to be located in the hippocampus was unclear, and it was hypothesized that they are the product of a second wave of CA1 neuron migration during development (Zhang et al., 1995), or merely CA1 pyramidal neurons that failed to form a layer (Chen and Hillman, 1986). Recent evidence that MAM causes structural abnormalities, such as rosettes and pronounced vacuolization in radial glial fibers (Zhang et al., 1995), may provide a more plausible explanation for the appearance of these heterotopias.

In addition to hippocampal heterotopias, injection of MAM on E15 causes the neocortex to be reduced in thickness, with only layers 5 and 6 remaining intact (Jones et al. 1982; Ashwell, 1987). Presumably, neurons destined to form the deeper layers of the cortex are fully developed and have completed their migration by E15, and so MAM injected at this time only exerts its antiproliferative effects on neuroblasts

destined for the more superficial layers (Cattabeni and Di Luca, 1997). This phenomenon, in combination with the observations that MAM causes lesions in radial glia, suggests that the ectopic cells in area CA1 of the MAM-treated hippocampus may not be hippocampal neurons at all. Interestingly, neurons in these heterotopias share a similar pattern of calbindin and parvalbumin expression with supragranular neocortical cells, and do not express limbic-associated membrane protein, a molecule specific to the hippocampus (Chevassus-au-Louis et al., 1998a). As well, these displaced neurons fail to express mRNAs that are normally present in CA1 pyramidal cells, but do express *Id-2*, an mRNA predominately expressed by layer 2-3 neocortical neurons (Castro et al., 2002), and possess a functional synaptic connection with the supragranular neocortex (Chevassus-au-Louis et al., 1998b; Baraban et al., 2000). Further, heterotopias only appear in the hippocampus postnatally (Singh, 1977; Chevassus-au-Louis et al., 1998a), indicating a postnatal migration pattern for the ectopic neurons, which are generated between E16 and E20. These properties reflect those of layer 2-3 neocortical pyramidal cells (Chevassus-au-Louis et al., 1998a). The observation that CA1 heterotopias are largely avoided by the surrounding Schaffer/commissural network of the stratum radiatum (SR) also suggests that these ectopic neurons are intrinsically foreign to their environment (Singh, 1978; Chevassus-au-Louis et al., 1999a). Clearly, there is a growing pool of evidence suggesting that the ectopic neurons in the hippocampi of MAM-treated rats were originally fated to reside in the neocortex, and damage to radial glial fibres by MAM may have led to their aberrant migration.

The observation that heterotopic neurons in the hippocampus are integrated into both their fated (neocortical) network, as well as the surrounding hippocampal network (Chevassus-au-Louis et al., 1998b; Baraban et al., 2000), thus forming a functional bridge between the limbic system and the neocortex, is of particular interest in the study of epilepsy. Despite evidence that the surrounding Schaffer-commisural network avoids the dysplastic area, hippocampal paroxysmal activity generated by application of the $GABA_A$ receptor antagonist, bicuculline, has been shown to spread directly to the neocortex via the heterotopia in MAM-treated but not in control animals (Chevassus-au-Louis et al., 1998b), thereby causing rapid generalization of epileptiform activity. In vitro, hippocampal slices from MAM rats demonstrate epileptiform activity in the presence of elevated extracellular potassium, while control slices do not (Baraban and Schwartzkroin, 1995). Also, adult MAM rats are more susceptible to seizures induced by convulsing agents such as kainic acid (Germano and Sperber, 1997, 1998), flurothyl (Baraban and Schwartzkroin, 1996), hyperthermia (Germano et al., 1996), and kindling (Chevassus-au-Louis, 1998c), and they exhibit an anticonvulsant-pharmacoresistant profile that matches clinical pediatric epilepsies (Smyth et al., 2001). Certainly, the brains of animals with cortical malformations due to prenatal exposure to MAM have been shown to be hyperexcitable, with reduced seizure threshold, in comparison to those of untreated animals, and have therefore become a well-known model by which to study the relationship between NMDs and epilepsy.

The increased seizure susceptibility seen in these animals may be due to a number of factors. In vitro electophysiological recordings aimed at determining the cellular or synaptic mechanisms contributing to epileptogenesis in MAM rats have demonstrated that heterotopic clusters of neurons in hippocampal area CA1 of the hippocampi are capable of generating synchronized epileptiform discharges in the absence of synaptic input from CA3 (Chevassus-au-Louis et al., 1999b). This hyperexcitability may be due, in part, to the abnormally low expression of GluR2 receptor subunits that has been observed in heterotopias of MAM rats (Germano et al., 1996). As the AMPA/kainite GluR2 subunit is responsible for preventing calcium entry into neurons (Rafiki et al., 1998), reduced levels of GluR2 may increase the amount of calcium entering a cell and cause excessive neurotransmitter release. As well, *in situ* hybridization and immunohistochemical analysis have revealed markedly reduced expression of Kv4.2 (A-type) potassium channel subunits in heterotopic neurons, which may lower the seizure threshold in MAM rats (Castro et al., 2001). As catecholamines are known to modulate neuronal spiking properties, and regulate the spread of paroxysmal activity in the epileptic brain (Chauvel et al., 1986), the hyperdensity of catecholaminergic fibers in all cortical structures of MAM rats that has been described by Johnston and Coyle (1982) may also contribute to epileptiform activity. Although these abnormalities all lead to hyperexcitability in the MAM brain, an unusual observation in these animals is that GABAergic innervation of the hippocampal heterotopias is actually enhanced (Calcagnotto et al., 2002), thus increasing functional inhibition in the area. However, it is well established that MAM rats do not exhibit spontaneous seizures (Chevassus-au-Louis et al., 1999b; Baraban

et al., 2000), and so elevated inhibitory neurotransmitter in dysplastic areas may act as a compensatory response to prevent epileptogenesis.

The Role of Neuropeptide Y in Epilepsy

The neuropeptide Y (NPY) family was initially discovered in the form of pancreatic polypeptide (PP), when it was found as a byproduct while Kimmel and coworkers (1975) isolated insulin from the chicken pancreas. Subsequently, Tatemoto and Mutt (1980) reported that porcine brain and gut contain large amounts of a peptide resembling PP. This peptide, isolated from the gut, was named peptide YY (PYY) because both its N- and C- terminal amino acids are tyrosine (Y being the single-letter abbreviation for tyrosine). Soon after, Tatemoto (1982) described the peptide in the brain, and named it neuropeptide Y (NPY). NPY, PP, and PYY are very closely related, composed of 36 amino acids each and sharing considerable amino acid homology, amidated C-terminal ends, and the presence of a large number of tyrosine residues including both ends of the molecule (Larhammar, 1996). All members of the NPY family act upon the same family of receptors, and so it is recommended that the receptors for NPY, PYY, and PP be classified together as NPY receptors (Michel et al., 1998).

The most abundant neuropeptide in the mammalian brain (Chronwall et al., 1985), NPY is primarily synthesized and released by neurons, and is widely distributed in the central (CNS) and peripheral (PNS) nervous systems (O'Donohue et al., 1985). NPY is often co-localized with classical neurotransmitters in nerve terminals, particularly catecholamines in the PNS (Sundler et al., 1993), and is preferentially released from neurons under conditions of high frequency stimulation and elevated neuronal activity (Bartfai et al., 1988; Hokfelt, 1991). Common physiological effects attributed to NPY

include stimulation of food intake and inhibition of anxiety in the CNS (Stanley and Leibowitz, 1985; Colmers and Bleakman, 1994; Wettstein et al., 1995), presynaptic inhibition of neurotransmitter release in the CNS and the PNS (Colmers et al., 1987; Lundberg, 1996), vasoconstriction (Michel and Rascher, 1995), inhibition of insulin release (Wang et al., 1994), regulation of gut motility (Sheikh, 1991), and gastrointestinal and renal epithelial secretion (Playford and Cox, 1996). These effects are mediated by interaction of NPY with a number of different receptor types.

All known NPY receptors belong to the large superfamily of G-protein-coupled, heptahelical receptors (GPCRs) (Michel et al., 1998). Initially, two subtypes of NPY receptors were proposed on the basis of the affinity of the 13-36 fragment of the NPY protein, and they were named receptors Y1 and Y2 (Wahlestedt et al., 1986). The Y1 receptor requires the entire NPY molecule for activation, whereas Y2 is selectively stimulated by the long (3-36) C-terminal NPY fragment. Both receptors Y1 and Y2 are equally responsive to NPY and PYY, but the observation that some actions of NPY cannot be performed by PYY led to the classification of a third receptor subtype (Y3) (Balasubramaniam et al., 1990). Although the Y3 receptor has not yet been cloned (Michel et al., 1998), it seems to be exclusively sensitive to NPY, and is typically found in the brain stem, heart, and adrenal medulla (Balasubramaniam et al., 1990). Subsequently, two more NPY receptors were classified: Y4, which preferentially binds PP (Michel et al., 1998), and Y5 (Gerald et al., 1996), with Y5 receptors raising considerable interest as mediators of NPY-stimulated food intake and leading to discussions of the possible existence of even more NPY receptor

subtypes, with Y5-like recognition features, in peripheral tissues (Biscoff and Michel, 1999). The most recent subtype to be cloned is y6 (Gregor et al., 1996; Weinberg et al., 1996), denoted with a lower case 'y' due to its poor conservation between species and the fact that a physiological correlate has not yet been described (Michel et al., 1998). Numerous selective agonists, and a much shorter list of antagonists, to the NPY receptor subtypes have been developed, providing useful pharmacological tools to study the role of NPY and NPY receptor subtypes in physiological function (Michel et al., 1998).

Interestingly, the Y2 receptor is the most abundant in the brain (Aicher et al., 1991), with its highest concentrations found in the hippocampus (Aicher et al., 1991; Dumont et al., 1990). NPY itself is particularly localized to GABA interneurons in the stratum oriens of Ammon's horn (Kohler et al., 1986; Gruber et al., 1994), and Y2 receptors are present in the strata oriens and radiatum of CA1 and CA3 (Dumont et al., 1993). In this structure, NPY acts presynaptically to inhibit excitatory transmission at glutamatergic stratum radiatum-CA1 and mossy fiber-CA3 synapses (Colmers et al., 1985, 1987, 1988, 1991), by reducing calcium influx at the presynaptic terminal (Toth et al. 1993). The NPY receptors responsible for this presynaptic inhibition match the previously classified agonist profile for the Y2 receptor subtype (Colmers et al., 1991). Because an association of the hippocampal formation with epileptic activity has been well established (Schwartzkroin, 1994), the role of NPY, an inhibitory neurotransmitter, in controlling hyperexcitability in this structure is of particular interest.

The first indications that NPY may be involved in seizure modulation came when increased levels of NPY mRNA were observed in limbic neurons after acute seizures induced by kainic acid (Marksteiner et al., 1989; Sperk et al., 1992; Gruber et al., 1994), electroconvulsive shock (Stenfors et al., 1989), or hilar lesion (White and Gall, 1987), and in spontaneously epileptic rats (Sadamatsu et al., 1995). Seizures have been shown to elevate NPY levels in granule cells and mossy fibers that do not normally contain NPY, as well as in the inhibitory interneurons of the Schaffer-commisural network. However, the level of neuronal excitation required for inducing NPY synthesis in granule cells is much higher than that needed in interneurons (Gruber et al., 1994). The mechanism behind seizure-induced NPY synthesis is unclear, but it may be related to the observation that brain-derived neurotrophic factor (BDNF) infusion into the hippocampus also increases NPY expression in interneurons (Nawa et al., 1994). Another factor that may affect the regulation of NPY expression is excitatory glutamatergic transmission, as stimulation of metabotropic glutamate receptors increases NPY levels in granule cells (Schwarzer and Sperk, 1998), and ionotropic glutamate receptors have been shown to be involved in increasing NPY synthesis in interneurons (Gruber et al., 1994). Presumably, the increase in NPY synthesis that occurs after seizure activity functions as an endogenous anticonvulsant mechanism, by upregulating the inhibition of excitatory transmission in the hippocampus.

Further evidence that NPY is involved in epilepsy and seizure modulation lays in observations that NPY receptor binding and expression is enhanced by epileptiform activity. In particular, radioligand binding to Y2 receptors was elevated within 6 h of kainic acid- or kindling-induced seizures in the strata oriens and radiatum of the hippocampus (Roder et al., 1996; Gobbi et al., 1998; Schwarzer et al., 1998). The elevated binding is due to an increased affinity of Y2 receptors for the ligand on presynaptic terminals of Schaffer collateral fibers, rather than an increase in the number of receptors present (Schwarzer et al., 1998). This increased affinity is associated with an enhanced efficacy of Y2-receptor ligands to inhibit glutamate release in the hippocampus (Greber et al., 1994; Schwarzer et al., 1998). Additionally, 24 h and 30 days after seizures, Y2 receptor binding and mRNA levels are enhanced in the dentate gyrus (Gobbi et al., 1998; Schwarzer et al., 1998), indicating the presence of newly synthesized Y2 receptors on mossy fiber nerve terminals. However, the regulation of NPY receptor expression with regard to seizure activity is not well understood, and may not represent a beneficial physiological adaptation (Vezzani et al., 1999).

Perhaps the most convincing studies where NPY has been shown to modulate seizure activity are those in which electrophysiological and pharmacological means are employed. *In vitro*, the aforementioned inhibitory actions of NPY on glutamate release in the hippocampus have been shown to reversibly abolish epileptiform activity induced by picrotoxin, stimulus-train-induced bursting (STIB), and removal of extracellular magnesium, predominantly via Y2 receptors (Klapstein and Colmers,

1997). *In vivo*, neuronal release of NPY is enhanced in the hippocampus after limbic seizures are induced in rats with kainic acid (Vezzani et al., 1994), injection of NPY into area CA1 of the hippocampus reduces excitatory behaviors induced by picrotoxin (Smialowska et al., 1996), and intracerebral administration of NPY shortens the duration of afterdischarges elicited in the hippocampus by stimulation of the subiculum (Woldbye et al., 1996). These studies indicate that NPY itself possesses anticonvulsant activity, and suggest that NPY release may be a compensatory mechanism to reduce excitation in the hippocampus during a limbic seizure.

The development of NPY-deficient mice by Erickson et al (1996) allowed further exploration of the role of NPY in epilepsy. Interestingly, NPY knock-out mice exhibit spontaneous seizure activity and a reduced threshold for GABA antagonist pentylenetetrazol-induced seizures (Erickson et al., 1996). As well, kainic acid-induced limbic seizures are fatal in mice lacking NPY, but not in their wild-type littermates (Baraban et al., 1997). These data suggest that NPY plays an important role in regulation of hippocampal function during a condition of hyperexcitability, but Baraban et al. (1997) have shown that NPY may not be required for hippocampal function under normal conditions, as no electophysiological deficits have been observed in hippocampal slices from NPY-deficient mice.

Clearly, there is a relationship between NPY, Y2 receptors, and seizure activity in the hippocampus. The inhibition of glutaminergic excitatory neurotransmission by NPY, as well as its effect on epileptiform activity, suggests that a primary role of this

peptide in the hippocampus is to modulate glutamate release and reduce excitability. Further, the observation that NPY and Y2 receptor mRNA is upregulated in the hippocampus following seizure activity suggests that seizure-induced accumulation of NPY in this structure may serve as a self-limiting role to specifically reduce excitatory transmission during conditions of hyperexcitability. As well, recent work in our lab with a Y2-selective antagonist, BIIE0246, has shown that Y2 is the dominant NPY receptor in the rat hippocampus *in vitro*, mediating both the presynaptic inhibitory and anti-epileptic actions of NPY in this structure (El Bahh et al. 2002). It is possible that these findings, in combination with evidence that exogenously applied NPY has anticonvulsant actions in vivo (Woldbye, 1996, 1997), are relevant to human epilepsy as Y2 receptors are also prominently expressed in the human hippocampus (Jacques et al., 1997). However, further studies must be done before NPY receptors can become viable targets for anticonvulsant therapies.

The Present Study

In the present study, we attempt to combine the above three subjects into an exploration of the effects of NPY on synaptic transmission in the MAM model of cortical dysplasia. Because neuronal migration disorders and cortical dysplasias are a major cause of early-onset epilepsy (Taylor et al., 1971; Dunn et al., 1986; Barkovich and Kjos, 1992; Battaglia et al., 1997), and a clear connection has been made between NPY and seizure modulation (Vezzani et al., 1999), the effects, or lack thereof, of NPY on synaptic transmission in the dysplastic hippocampi of MAM rats may help explain how disorganized areas become epileptic foci. Using whole cell patch clamp recording, and an IR-DIC videomicroscopy system, the synaptic and intrinsic properties of neurons located in heterotpias of area CA1 of the hippocampi of MAM rats were examined, with particular attention paid to the effect of NPY on these ectopic cells. Our hypotheses were twofold: 1) the effect of NPY on excitatory synaptic transmission to heterotopic neurons is altered in some way, thereby contributing to the hyperexcitability of these cells, and 2) the intrinsic properties of hippocampal heterotopic neurons in MAM rats are similar to those of control layer 2-3 neurons of the supragranular neocortex, in accordance with previous studies. Electrophysiological and pharmacological studies of over 200 neurons have produced the data and conclusions discussed in the following chapter.

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Chapter II

NPY Sensitivity and Post-Synaptic Properties of Heterotopic Neurons in the Methylazoxymethanol Model of Malformation-Associated Epilepsy*

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Introduction

Neuronal migration disorders, in which newly born neurons fail to migrate correctly from the ventricular zone to their final neocortical positions, are often associated with neurological dysfunction. In children, for example, cortical disorganization resulting from a migration disorder can be associated with intractable forms of epilepsy, mental retardation, or autism (Friede, 1975; Aicardi, 1994; Palmini, 2000). Because seizures result from the abnormal electrical discharge of a group of neurons, much effort has been directed toward studying clusters of disorganized neurons (i.e. neuronal heterotopia). Interestingly, clinical studies suggest that disorganized brain regions generate seizure activity (Raymond et al., 1994) and surgical resection of this tissue is often an effective form of seizure control (Palmini et al., 1991a,b).

A strong clinical correlation between migration disorders and epilepsy spurred the development of several animal models in which to study epileptogenesis in the disorganized brain (Chevassus-au-Louis et al., 1999). One such model utilizes prenatal exposure to the teratogenic, DNA methylating agent, methylazoxymethanol (MAM) (Nagata and Matsumoto, 1969). MAM injection on gestational day 15 in rats results in diffuse cortical malformations, including microcephaly, heterotopias in area CA1 of hippocampus, and loss of lamination (Singh, 1977; Chen and Hillman, 1986). These animals exhibit many of the anatomical/molecular properties of human cortical dysplasia, and are significantly seizure-susceptible (Baraban and Schwartzkroin, 1996; Germano and Sperber, 1997; Chevassus-au-Louis et al., 1999a, b; Colacitti et al., 1999). Similar to clinical studies, hippocampal heterotopias in an experimental model are of particular

interest as a potential site of seizure generation. Recent work suggests that hippocampal heterotopic neurons (i) are capable of independent seizure genesis (Baraban et al., 2000), (ii) exhibit hyperexcitable firing activity and a loss of functional, A-type potassium channels (Castro et al., 2001) and (iii) are most similar, both in molecular and electrophysiological properties, to neocortical neurons (Chevassus-au-Louis et al., 1998; Castro et al., 2002).

Although the hyperexcitability of hippocampal heterotopic neurons in the MAM model is now well established, synaptic physiology within a heterotopia has been little studied and virtually nothing is known about modulation of excitation at heterotopic synapses. Here we examined the actions of neuropeptide Y (NPY), a potent, endogenous modulator of hippocampal excitability (Colmers et al., 1988), on excitatory synaptic inputs to heterotopic cells in hippocampi from MAM-treated rats, as well as on normotopic CA1 pyramidal neurons (MAM-treated and control untreated rats). While NPY inhibited stratum radiatum (SR) excitatory synaptic input to heterotopic neurons, it had little effect on intra-heterotopic excitation. Furthermore, heterotopic neurons shared few membrane and synaptic properties with CA1 pyramidal cells, but were virtually indistinguishable from neocortical layer 2-3 neurons. These data support other evidence (Chevassus-au-Louis et al., 1998; Castro et al., 2002) suggesting that MAM-induced heterotopic neurons in area CA1 of the hippocampus were fated to become layer 2-3 neocortical neurons.

Methods

Preparation of Slices

Pregnant Sprague-Dawley rats were injected i.p. with 25 mg/kg of MAM-Acetate (NCI Chemical Carcinogen, Kansas City, MO) on day 15 of gestation. Male and female offspring (17–35 days old) were decapitated according to a protocol approved by the Health Sciences Laboratory Animal Welfare Committee of the University of Alberta. The brain was rapidly removed and placed in ice-cold (2-4°C), carbogen (95% O_2-5% CO_2)-saturated slicing medium containing (in mM) 118 NaCl, 3 KCl, 1.3 $MgSO_4$, 1.4 NaH_2PO_4, 5 $MgCl_2$, 26 $NaHCO_3$, 1.5 $CaCl_2$, 10 glucose, and 1 kynurenic acid (to block glutamate-mediated excitoxicity). The brain was hemisected sagitally, and the cerebellum and frontal lobe removed. Blocked tissue was glued to the base of a plexiglass slicing chamber. Transverse slices (300 μm) containing hippocampus and neocortex were cut with a vibratome (TPI, St. Louis, MO), and immediately transferred to a holding chamber containing carbogen-saturated artificial cerebrospinal fluid (aCSF) consisting of (in mM) 124 NaCl, 3 KCl, 1.3 $MgSO_4$, 1.4 NaH_2PO_4, 26 $NaHCO_3$, 2.5 $CaCl_2$, and 10 glucose. Slices were held at 32°C for 30-60 minutes, then stored at room temperature for up to 7 hours.

Electrophysiological Recordings

Individual slices were transferred to a glass-bottomed submersion-type recording chamber, anchored with a platinum "harp," and continuously perfused with oxygenated aCSF at 34-36°C. Neurons were visually identified using an IR-DIC videomicroscopy system, as described by Ho et al. (2000). Whole-cell recordings were performed with

patch electrodes (3-6 MΩ) pulled from borosilicate glass capillary tubing, filled with an intracellular solution consisting of (in mM): 125 K-gluconate, 2 KCl, 5 HEPES, 5 MgATP, 0.3NaGTP, 5 EGTA, 0.1 BAPTA, 10 creatine phosphate, and 3.0 mg/ml biocytin (pH 7.2; 292-298 mOsm). Once a seal (>2 GΩ) was formed, the patch was ruptured to gain access to the cell (15-40 MΩ). Whole cell experiments were performed on pyramidal neurons in hippocampal heterotopias, in stratum pyramidale of area CA1 of the hippocampus, and in layer 2-3 of the neocortex overlying the hippocampus in MAM-treated or normal rats. Data were taken only from neurons whose resting membrane potential was stable and negative to -55 mV. Once a stable membrane potential had been observed, neurons were held in voltage-clamp, near their resting potentials (-65 mV for CA1 neurons, and –75 mV for heterotopic and cortical pyramidal cells, except where noted) for the duration of the experiments. Excitatory post-synaptic currents (EPSCs) were evoked via a bipolar, sharpened tungsten stimulating electrode placed in stratum radiatum (SR) of area CA1, within the heterotopia, or in layer 1 of neocortex. A paired-pulse stimulus protocol (1-20V, 300 μs, 50-ms interstimulus interval) was delivered to the stimulating electrode from a stimulus isolation unit (IsoFlex, AMPI, Jerusalem). The intensity of the stimulus was adjusted until a submaximal and stable synaptic current was evoked. In most cases, a voltage step (50 ms, 10-20 mV negative to rest) was applied to the neuron during the protocol, after the synaptic responses had subsided, to monitor for changes in access resistance (Ho et al., 2000).

Passive postsynaptic membrane properties were also routinely examined in virtually every recording with a slow (2.8 s, 60 mV), positive-going voltage ramp starting 40 mV negative to rest, and with a family of 100-ms voltage steps, varying, from -40 mV to +20

mV relative to the resting membrane potential in 10 mV increments, with a 2s interval between each step. All whole cell currents were recorded using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) used in the continuous single electrode voltage-clamp mode. Data were acquired and membrane potential controlled using pClamp 8 (Axon Instruments). Drugs were applied via the bath.

Materials

NPY was purchased from Dr. S. St.-Pierre (Peptidec Technologies, Montreal, QC, Canada); 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (NBQX) was purchased from Research Biochemicals International (RBI, Natick, MA); 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride (ZD 7288) was purchased from Tocris (Ellisville, MO); Creatine phosphate was purchased from Boeringer (Mannheim, Germany); Cesium chloride (Cs_2Cl) and barium chloride (BaCl) were purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals used in slicing medium and aCSF were obtained from BDH (Toronto, ON, Canada) and all other chemicals were obtained from Sigma (St. Louis, MO).

Analysis

Data were analyzed using pClamp 8 (Axon Instruments) and GraphPad Prism 3.02 software (GraphPad, San Diego, CA). Graphs were made using Axum 5.0 (Mathsoft, Cambridge, MA). Neurons were used as their own controls for statistical purposes. Data on NPY and ion channel blockers are only from experiments in which the effects reversed substantially during washout. Numerical data are presented as means ± SEM.

Statistical comparisons of the results' significance versus zero were made using a Student's unpaired *t* test, and comparisons between cell types were done using a student's paired *t* test.

Histochemistry

The procedure, with some modifications, was similar to the biocytin visualization procedure described elsewhere (Schiller et al., 1997). Briefly, slices with biocytin-filled neurons were fixed in ice-cold, 4% paraformaldehyde in 100 mM phosphate-buffered saline (PBS). Thereafter, the slices were rinsed in PBS, then in PBS containing 1% H_2O_2 to quench endogenous peroxidases. Sections were rinsed thoroughly in PBS and then 2% Triton X100 in PBS for one hour to increase the penetration of reagents. Slices were then incubated for two hours in avidin-biotinylated horseradish peroxidase according to the manufacturer's protocol (ABC-Elite, Vector Labs, Peterborough, UK), and then rinsed thoroughly in PBS. Cells were visualized using diaminobenzidine (0.05%) in 0.01% H_2O_2 PBS, and the reaction was quenched by rinsing again in standard PBS. Finally, slices were mounted on slides with an aqueous medium, and photographed with a color digital camera (Dage DC330, Dage-MTI, Michigan City, IN).

Results

Cell Morphology

Results are based on recordings from more than 100 heterotopic neurons and 35 normotopic CA1 neurons from MAM-treated animals, 43 CA1 pyramidal cells and 43 layer 2-3 neocortical neurons from untreated rats. We did not include physiological data from the recordings of neocortical neurons from MAM-treated rats in this study, as the MAM treatment used interferes with the formation of neocortical layers 2-4 (Jones et al., 1982), thus making it difficult to unambiguously assign a neuron to a specific neocortical layer. As previously described (Chen and Hillman, 1986; Baraban et al., 2000), brains from MAM-treated rats were severely microcephalic, with enlarged ventricles and significantly smaller neocortex and hippocampi compared with untreated animals.

To examine the morphology of heterotopic neurons in MAM-treated rats, biocytin was included in the patch pipette. In accordance with previous Golgi staining studies (Singh, 1980), biocytin-filled heterotopic neurons were smaller than normotopic CA1 pyramidal neurons, with abnormally branched dendrites and deformed dendritic shafts (Figs. 1*A-D*). Normotopic CA1 pyramidal neurons from MAM-treated rats appeared smaller in size, and exhibited a greater degree of dendritic branching, compared with CA1 pyramidal neurons from age-matched controls (Figs. 1*E* and *G*). However, it is important to note that the altered dendritic morphology in CA1 neurons from MAM-treated rats does not dramatically affect physiological function, as the basic intrinsic properties of these cells do not differ substantially from those of untreated animals (Baraban and Schwartzkroin, 1995). Biocytin-filled neocortical neurons from MAM rats appear to have increased

dendritic branching compared with control layer 2-3 neocortical pyramidal cells and the dendrites of these cells were not aligned in an orderly fashion (Figs. 1*F* and *H*).

Neuropeptide Y actions in the MAM brain

Hippocampal heterotopic neurons are hyperexcitable and a potential source of seizure genesis in MAM-treated rats (Baraban et al., 2000; Castro et al., 2001). NPY inhibits excitatory synaptic transmission in the hippocampus (Colmers et al., 1988) and thereby exerts a powerful antiepileptic role in rodents (Colmers et al., 1988; Baraban et al., 1997; Klapstein and Colmers, 1997; Bindokas et al., 1998; Marsh et al., 1999; Vezzani et al., 1999). In the present study, we examined the modulatory effect of NPY in acute hippocampal slices from MAM- and untreated control rats. 1 μM NPY potently inhibited excitatory postsynaptic currents (EPSCs) elicited by stimulation in stratum radiatum (SR) both in normal CA1 pyramidal (Control: 85.7 ± 1.78%, n=11, $p<0.0001$) and normotopic CA1 pyramidal neurons (MAM: 72.7 ± 4.15%, n=8, $p<0.0001$) (Figs. 2*A, B,* and *E*). The same concentration of NPY had a significantly smaller, yet still significant, effect on EPSCs evoked in heterotopic cells by SR stimulation (MAM: 54.1 ± 4.10%, n=16, $p<0.0001$) (Figs. 2*C* and *E*). However, when the stimulating electrode was placed within the heterotopia, 1 μM NPY had no effect on EPCS's evoked in 8 out of 19 heterotopic neurons, and only weakly inhibited EPSCs in the remaining 11 cells (10.96 ± 1.38%, n=11, $p<0.0001$) (Figs. 2*D* and *E*). Thus, intra-heterotopic excitatory connections are far less responsive to the actions of NPY than are extra-heterotopic, presumably Schaffer collateral, inputs. It is important to note that eliciting EPSCs in heterotopic neurons from the stratum radiatum was only successful approximately 40% of the time, presumably

because the fibres of the SR tend to avoid the heterotopia, as has been shown previously using carbocyanine tracing (Chevassus-au-Louis et al. 1999b). However, EPSCs that were evoked in heterotopic neurons from the SR were done so using a similar stimulus intensity as was needed when the stimulating electrode was placed within the heterotopias.

Characterization of EPSC responses in the MAM brain

To further characterize the synaptic properties of hippocampal heterotopic neurons, we examined the pharmacological and biophysical properties of evoked glutamatergic EPSCs. To examine functional NMDA-mediated synaptic responses on heterotopic neurons, we perfused slices with an NMDA receptor antagonist, D-2-amino-5-phosphopentanoic acid (APV). At a holding potential of –45 mV, virtually all NMDA receptors are free from the blockage by magnesium that occurs at more negative membrane potentials (Nowak et al., 1984). In neurons held at –45 mV, we found that 50 μM APV inhibited the EPSCs on layer 2-3 cortical neurons (52.6 ± 6.23%, n=7, $p>0.001$) and CA1 pyramidal cells (59.1 ± 8.56%, n=7, $p>0.007$) from normal animals significantly more than its inhibition of EPCSs on hippocampal heterotopic neurons (22.3 ± 1.78%, n=7) (Fig. 3*B*). Addition of 3 μM NBQX, an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-selective antagonist (Sheardown et al. 1990) following APV caused a further reduction of EPSC amplitude to approximately 7% of pre-drug levels for all three cell types (data not shown). Thus, the glutamatergic EPSCs recorded here in these experiments were comprised mainly of NMDA and AMPA components.

Qualitative observations of evoked synaptic responses suggest that EPSCs in heterotopic or layer 2-3 neocortical neurons were quite fast compared with EPSCs in normotopic CA1 pyramidal neurons from both MAM and control animals (Figs. 4*A-D*). To compare this quantitatively, we determined decay time constants of the EPSC (τ_{EPSC}) for each cell type, using a standard exponential fitting model. At a holding potential of –75 mV, there was no significant difference between the τ_{EPSC} of heterotopic (4.8 ± 0.20 ms, n=26) and layer 2-3 neocortical neurons from control animals (5.1 ± 0.26 ms, n=23, p>0.39) (Fig. 4*E*). However, the τ_{EPSC} in control CA1 pyramidal cells held at –75 mV was much greater than in the other cell types (7.9 ± 0.95 ms, n=16, p=0.0001), and was not significantly different than those recorded in normotopic CA1 pyramidal neurons (7.4 ± 0.71 ms, n=5, p>0.8) (Fig. 4*E*). When neurons were held at –65 mV, there was still no significant difference between the τ_{EPSC} in heterotopic and layer 2-3 pyramidal neurons (4.3 ± 0.30 ms, n=21 vs. 4.9 ± 0.30 ms, n=17, p>0.15), and they remained significantly smaller than either the τ_{EPSC} of normal CA1 cells (7.864 ± 0.4091 ms, n=22, p<0.0001), and normotopic CA1 pyramidal neurons (MAM: 8.1 ± 0.68 ms, n=12), which did not significantly differ from one another. (p>0.95).

Comparisons of membrane properties in pyramidal cells from MAM-treated and normal animals

During the previous experiments on synaptic actions, we routinely observed postsynaptic properties in some detail to determine whether any changes in synaptic responses with NPY application might be explained by alterations in postsynaptic properties. The first

noticeable difference between heterotopic and normotopic CA1 neurons from MAM-treated animals, was that heterotopic neurons rested at a significantly more negative potential than did either normotopic CA1 neurons (-74.6 ± 0.42 mV, n=102, vs. -64.9 ± 0.42, n=35, $p<0.0001$) or CA1 neurons from control animals (-64.4 ± 0.54, n=43, $p<0.0001$). However, the resting potentials of layer 2-3 neocortical neurons were not significantly different (-74.19 ± 0.57, n=43) from those of heterotopic neurons ($p>0.5$).

No evidence was found for an effect of NPY on postsynaptic properties in any neurons tested, consistent with earlier reports (Colmers et al., 1987, 1988; McQuiston and Colmers, 1996). However, we did observe differences in membrane steady-state current-voltage relationships between the different types of neurons studied here. These differences prompted a systematic comparison of the postsynaptic properties of neurons from MAM-treated and control animals.

First, we examined steady-state, current-voltage relationships using a slow voltage ramp protocol. As the membrane potential became more negative in heterotopic and neocortical neurons, the slope of the current-voltage response progressively increased. By contrast, the same protocol elicited a nearly linear response in CA1 pyramidal neurons (Figs. 5*A-C*). Second, we measured the chord conductance between –115 and –100 mV in voltage-clamped neurons. Measurements of chord conductance demonstrated that conductance in normal CA1 pyramidal neurons is relatively low in this voltage region (0.12 ± 0.01 nS, n=22) while it was significantly greater in heterotopic (0.24 ± 0.02 nS,

n=22, $p<0.0001$) and layer 2-3 neocortical neurons (0.20 ± 0.02 nS, n=13, $p<0.0019$) (Fig. 5*D*).

The increased conductance observed for heterotopic and cortical neurons at more negative potentials during the slow voltage ramp (Fig. 6*A*) is consistent with the presence of an inwardly rectifying potassium current, K_{IR}, (Williams et al., 1988; Hwa and Avoli, 1991; Hutcheon et al., 1996). Because K_{IR} can be inhibited equally well with Ba^{2+} or Cs^{+} (Williams et al., 1988), we next tested the effects of these compounds on the steady-state membrane conductance. Cs^{+} (2 mM) and Ba^{2+} (50 μM) both strongly reduced membrane conductance in heterotopic and layer 2-3 neocortical neurons, particularly at potentials negative to –90 mV (Figs. 6*B-C*). By contrast, although Cs^{+} strongly reduced membrane conductance at all potentials in CA1 pyramidal cells, Ba^{2+} only had a small effect at potentials negative to –90 mV (Fig. 6*B-C*).

Inwardly rectifying potassium currents are potentiated by increases in extracellular K^{+} (Pennefather and DeCoursey, 1994). Therefore, elevating the extracellular potassium to 6 mM increased K_{IR} on heterotopic and neocortical neurons. In the presence of 6 mM K^{+} the membrane conductance increased relative to control K^{+} in all three cell types, but increased the conductance most at –110 in heterotopic and layer 2-3 cells. Cs^{+} reduced the membrane conductance in all three types of cells, having an effect at every membrane potential in CA1 pyramidal neurons (-110 mV: $34.1 \pm 9.72\%$ inhibition, n=9; -60 mV: $19.5 \pm 7.42\%$ inhibition, n=9), and only affecting the conductance of heterotopic and layer 2-3 neocortical cells at potentials more negative than –90 mV, and –80 mV,

respectively (HET at –110 mV: 83.9 ± 8.27 % inhibition, n=6; COR at –110 mV: 98.8 ± 5.36% inhibition, n=11). Conversely, in 6 mM K^+, Ba^{2+} does not significantly alter membrane conductance in CA1 pyramidal neurons, but has a strong inhibitory effect on the steady-state conductance of both heterotopic and cortical neurons at –80 mV and below (HET at –110 mV: 75.2 ± 8.73% inhibition, n=6; COR at –110 mV: 60.4 ± 6.28% inhibition, n=11).

To further compare the neuronal properties of these cell types, a series of 100-ms voltage-clamp steps was applied from a holding potential of -75 mV. At a step to –115 mV, an inward relaxation was observed, possibly consistent with the presence of a hyperpolarization-activated cation current, I_h (Halliwell and Adams, 1982; Mayer and Westbrook, 1983; Pape, 1996). However, comparison of the time constant of this current relaxation at –115 mV (τ_H) showed that heterotopic and layer 2-3 neocortical neurons exhibited fast inward currents (MAM: 13.8 ± 0.26 ms, n=26 vs. Control: 15.2 ± 0.71ms, n=21) that did not differ significantly ($p>0.1$) (Fig. 7). Conversely, CA1 pyramidal neurons exhibit a much slower, more prominent inward relaxation (36.6 ± 1.19 ms, n=29, $p<0.0001$) (Fig. 7).

The strong inhibition of steady-state conductance by Ba^+ in heterotopic and layer 2-3 neurons suggests a very small I_h in these cells (Pape, 1996). We thus examined the effect of a selective I_h blocker, ZD 7288 (Harris and Constantini, 1995; Gasparini and DiFrancesco, 1997), on membrane conductance using the voltage step protocol described above. Comparison of currents elicited by a voltage step to –115 mV, in the absence or

presence of ZD 7288 (50 μM), demonstrated a strong inhibition of I_h in CA1 pyramidal neurons by ZD 7288, but only a very minor effect on heterotopic and neocortical neurons (Fig. 8). By subtracting current traces taken before and after application of ZD 7288, we were able to isolate the net I_h (Fig. 8*A-C*), which we compared at –115 mV in each cell type. As expected from the results with Ba^{2+}, above, the amplitude of I_h in CA1 pyramidal cells was substantial (103.6 ± 9.81 pA, n=6) and significantly larger than the amplitude of I_h in heterotopic (12.0 ± 4.74 pA, n=8, $p>0.0005$) and layer 2-3 neocortical neurons (14.7 ± 4.07 pA, n=5, $p>0.004$) (Fig. 8*D*). Consistent with the hypothesis that heterotopic and neocortical neurons share similar intrinsic properties, the amplitudes of I_h measured for heterotopic and layer 2-3 pyramidal neurons were not significantly different ($p>0.7$). The lesser effect of ZD 7288 in the heterotopia and cortex indicates that a much weaker I_h is present in heterotopic and layer 2-3 pyramidal cells than in CA1 pyramidal cells, consistent with the effects of Ba^{2+} in the voltage ramp experiments.

Discussion

Neuronal heterotopias are frequently seizure foci in patients suffering from epilepsies associated with a cortical malformation (Palmini et al., 1991a). Here we show that heterotopic neurons in area CA1 of the hippocampus of MAM-treated rats differ from neighboring normotopic CA1 pyramidal neurons (or control CA1 pyramidal neurons) in their connectivity, synaptic function and ion channel and presynaptic receptor complement. Hippocampal heterotopic neurons in these animals most closely resemble layer 2-3 neocortical neurons, as suggested previously (Chevassus-au-Louis et al., 1998; Castro et al., 2002), consistent with the hypothesis that these animals model a neuronal migration disorder. These results add to our growing knowledge of how neurons function in a disorganized brain, suggest that a variety of physiological functions are altered when neurons migrate incorrectly, and implicate alterations in presynaptic NPY receptors as one source of hyperexcitability in experimental heterotopias.

Limited NPY susceptibility within heterotopias

NPY has a substantial inhibitory effect on the amplitudes of EPSCs elicited by SR stimulation in all neurons tested, including CA1 pyramidal cells of both MAM-treated and untreated animals, and heterotopic neurons. By contrast, NPY had only a very weak effect on inputs to heterotopic neurons when the stimulating electrode was placed within the heterotopia. This suggests that the excitatory inputs to heterotopic neurons have few, if any, NPY receptors on their terminals. The fibers of stratum radiatum have a powerful presynaptic response to NPY (Qian et al., 1997). While the origin of excitatory inputs within the heterotopia is unclear, evidence here and elsewhere suggests that the *en*

passant fibres of the stratum radiatum are not the exclusive source. Earlier studies using carbocyanine tracing indicate that stratum radiatum fibers avoid the neurons of a heterotopia (Chevassus-au-Louis et al., 1999b), consistent with such ectopic neurons expressing a complement of cell surface markers suppressing their integration within the hippocampus. This raises the possibility that a considerable number of excitatory inputs originate within the heterotopia. As the intra-heterotopic inputs appear to lack NPY receptors, poorly-regulated heterotopic-heterotopic excitation may contribute to epileptiform activity found in these disorganized cell regions (Baraban et al., 2000), and may also be a factor in human neuronal migration disorders where dysplastic areas frequently form epileptic foci (Palmini et al., 1991a).

Reduced NMDA Component in Heterotopic EPSPs

In the present study, we have shown that the NMDA receptor antagonist APV does not have as strong an inhibitory effect on the EPSCs of heterotopic neurons as it does on those of layer 2-3 cortical and CA1 pyramidal cells. The reduced effect of D-APV in hippocampal heterotopic neurons seen here agrees with studies showing a relatively minor NMDA component in EPSCs for dysplastic neurons in the freeze-lesion model of cortical dysplasia (Luhmann and Raabe, 1996; DeFazio and Hablitz, 2000). Although there is recent evidence that the expression of NMDA receptors in disorganized areas of MAM-treated brain tissue is qualitatively similar to that in controls (Rafiki et al., 1998), the proportion of NMDA receptor type 2B subunits is increased in cortical dysplasias of humans (Najm et al., 2000) and experimental animals (Rafiki et al., 1998; DeFazio and Hablitz ,2000), suggesting a role for this subunit in epileptogenicity. While the present

study shows that NMDA has a reduced role in the generation of EPSCs in heterotopic neurons of the MAM model, this increased expression of NR2B may play a part in the propagation of epileptiform activity in disorganized tissue. Also, while this reduced NMDA effect may seem inconsistent with the intrinsically hyperexcitable nature of these neurons (Baraban et al., 2000), a synaptic hyperexcitability mediated by AMPA-receptors has been reported in a chronic model of temporal lobe epilepsy (Lothman et al., 1995). The changes in NMDA (and AMPA) receptor complement in heterotopias of the MAM model must be more thoroughly examined.

Hippocampal Heterotopic Neurons Have Similar Properties to Layer 2-3 Cortical Neurons

While heterotopic neurons shared relatively few physiological properties with normotopic or normal CA1 neurons, most properties studied were indistinguishable from those of layer 2-3 pyramidal cells. For example, heterotopic and cortical neurons both rest at a membrane potential of approximately –75 mV, while CA1 pyramidal cells in MAM-treated and untreated rats rest at approximately –65 mV. Furthermore, the τ_{EPSC} of heterotopic and cortical neurons are similar, and far faster than that in normal or normotopic CA1 pyramidal cells, consistent with their sharing similar postsynaptic responses to excitatory inputs. Likewise, heterotopic and layer 2-3 cortical neurons exhibit an increased conductance at membrane potentials negative to –80 mV. This conductance was sensitive to both barium and cesium, and was enhanced in elevated extracellular potassium, consistent with an inwardly rectifying potassium current (I_{IR}) (e.g., Williams et al., 1988). In contrast, the steady-state current-voltage relationship of

CA1 pyramidal neurons is linear across all membrane potentials examined, but is affected by cesium, and not by barium, consistent with an inwardly rectifying cation current (I_h) that has previously been described in hippocampal (Halliwell and Adams, 1982) and other neurons (Pape, 1996). Consistent with this, the I_h-specific blocker, ZD 7288, suppressed a prominent current in CA1 neurons, but had little effect in heterotopic and layer 2-3 pyramidal cells. Interestingly, the fact that heterotopic neurons possess a relatively small I_h may be a contributing factor to the hyperexcitability observed in these cells (Baraban et al., 2000). I_h typically acts as a 'pacemaker' in most neurons (Pape, 1996) and blockage of I_h channels in pyramidal neurons has been shown to increase the rate of spike generation (Gasparini and DiFrancesco, 1997) and lead to the enhanced summation of EPSPs (Berger et al., 2001; Magee, 1999). A combination of increased action potential generation caused by EPSP summation and decreased latency between these action potentials, both consequences of a reduced I_h, may be involved in epileptogenesis in the MAM model of cortical dysplasia. It is also possible that the lack of a prominent pacemaker current such as I_h in human brain dysgeneses contributes to seizure generation in NMD patients.

The similarities observed in intrinsic membrane properties of heterotopic and cortical pyramidal cells, as well as the differences seen here between heterotopic and CA1 neurons, indicate that hippocampal heterotopic neurons in MAM-treated rats may actually have been fated to become layer 2-3 cortical neurons. Previous studies have demonstrated that heterotopic neurons express genes specific to neurons of neocortical layer 2-3 (Castro et al., 2002), and that heterotopic and neocortical supragranular neurons

have similar developmental features and neuronal firing properties (Chevassus-Au-Louis et al., 1998; Castro et al., 2002). These findings, in combination with the additional electrophysiological similarities described here, provide strong evidence that hippocampal heterotopic neurons in MAM-treated rats are displaced layer 2-3 cortical cells, rather than the product of a second wave of CA1 neuron migration during development as previously hypothesized (Zhang et al., 1995).

In addition to producing hippocampal heterotopiae, the prenatal MAM treatment used here causes the neocortex to be reduced in thickness, with only layers 5 and 6 intact (Jones et al., 1982). The alteration in dendritic morphology seen here in biocytin-filled cortical neurons implies a disorganization in the outer layers of the neocortex that may be a consequence of the loss of layers 2-4. These abnormalities are consistent with an absence of cells destined for layers 2-4 of the neocortex. Because MAM is cytotoxic to dividing neurons, and the layer 2-4 neurons undergo DNA synthesis in the neuroepithelium just prior to the MAM treatment used here (Jones et al., 1982), the derangement of layers 2-4 may be due to a selective loss of these neurons or the migration of some of these neurons into the hippocampus. Additionally, MAM treatment on gestational day 15 causes diffuse structural abnormalities in radial glial cells (Zhang et al., 1995), which normally provide guidance for neurons migrating to their final destinations (Sidman and Rakic, 1973; O'Rourke et al., 1992). Thus, surviving cortical neurons may begin migrating without the aid of radial glia, and locate aberrantly in the hippocampus. While MAM-treated rats have not yet been shown to exhibit spontaneous seizures (Baraban and Schwartkzroin, 1995; Germano and Sperber, 1997; Chevassus-au-

Louis et al., 1999), the abnormal migration of neurons in the MAM-model may provide a clue as to the etiology of neuronal migration disorders (NMDs). Nonetheless, it is tempting to speculate that, in the larger assemblages of dysplastic neurons that form human cortical dysplasias, the combination of enhanced intrinsic excitability, collateral excitation and insensitivity to NPY action may increase the propensity to express focal seizure activity.

Figures

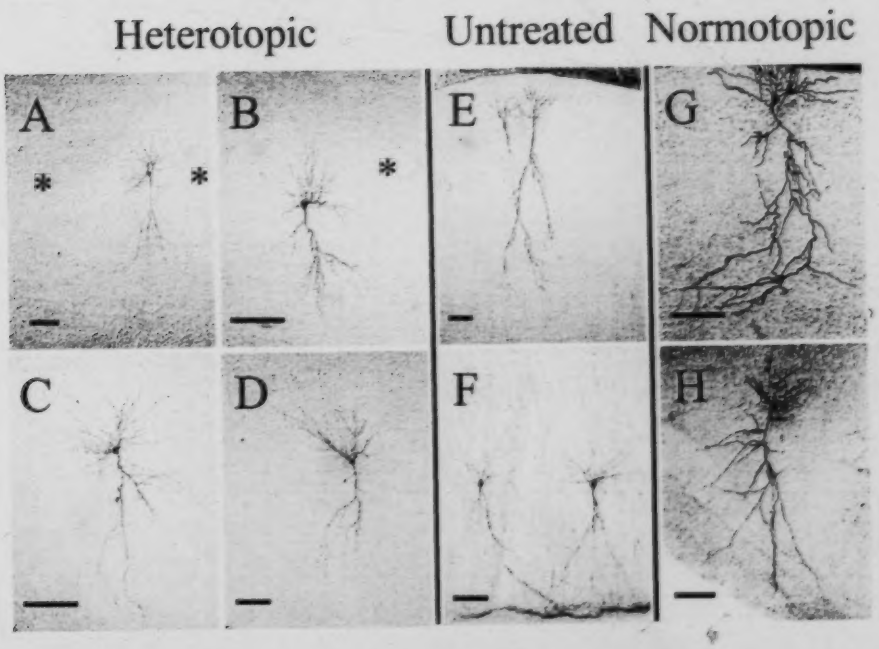

Figure 2-1. The effects of MAM on cell morphology in the hippocampus and layer 2-3 of the neocortex. *A-D*: biocytin-filled heterotopic neurons in area CA1 of the hippocampus. The asterisks in *A* and *B* indicate the points at which the CA1 cell body layers are interrupted by the heterotopiae. *E*: Two CA1 pyramidal neurons in an untreated animal. *F*: Two layer 2-3 corical neurons in an untreated rat. *G*: In an MAM-treated rat, CA1 pyramidal neurons exhibit abnormally branched, deformed dendrites. *H*: In MAM-treated animals, cortical neurons are disorganized and abnormally branched. Scale bars = 100μm in MAM-treated and untreated rats.

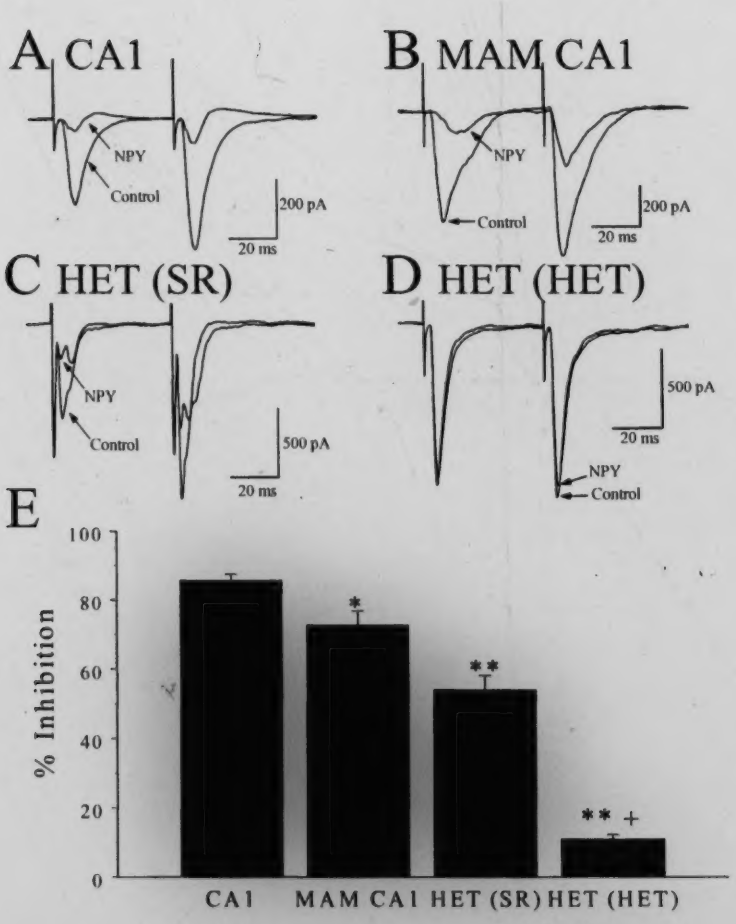

Figure 2-2. The effect of NPY (1μM) on exctitatory synaptic inputs to hippocampal neurons in control and MAM rats. *A*: NPY potently inhibits EPSCs elicited by stratum radiatum (SR) stimulation in CA1 pyramidal cells from untreated animals(CA1). *B*: SR-evoked EPSCs are also strongly inhibited by NPY in normotopic CA1 cells from MAM-treated animals (MAM CA1). *C* and *D*: In heterotopic neurons, NPY modestly inhibits the SR-evoked EPSCs (HET (SR)), but has either no effector only a very minor one in the intraheterotopically-evoked EPSCs (HET (HET)). *E*: effect of NPY (percent inhibition of peak EPSC amplitude) on the different synaptic inputs to the different cell types as illustrated in *A-D*, above. * - statistical significance in comparison with CA1 neurons from untreated rats, * - $P<0.015$; ** - $P<0.0001$.' + - statistical significance in comparison with SR inputs to heterotopic neurons, + - $P<0.0001$.

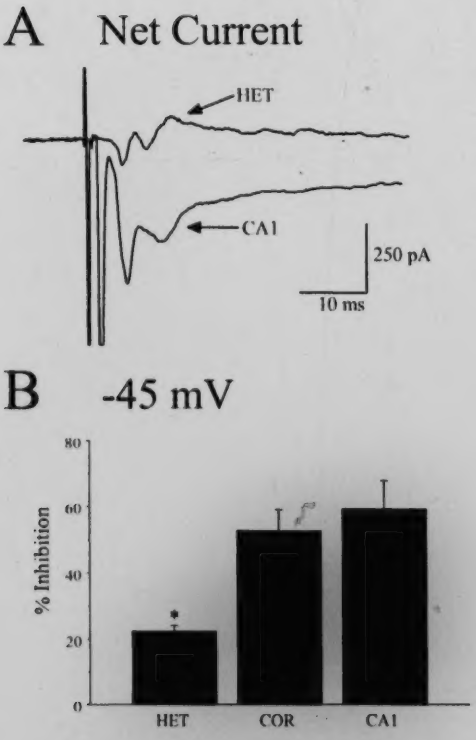

Figure 2-3. Effects of the NMDA-antagonist, APV, on EPSCs in different cell types. *A*: Net (digitally-subtracted) current abolished by NMDA receptor blockade in SR-evoked EPSCs in heterotopic neurons (HET) and in CA1 pyramidal cells from untreated rats (CA1). *B*: Average reduction in peak EPSC amplitude caused by APV application in heterotopic neurons (HET), and in cortical layer 2-3 (COR) evoked from layer 1, and CA1 pyramidal cells (CA1) from untreated animals. * - statistical significance in comparison with cortical neurons form untreated animals, * - $P<0.0013$.

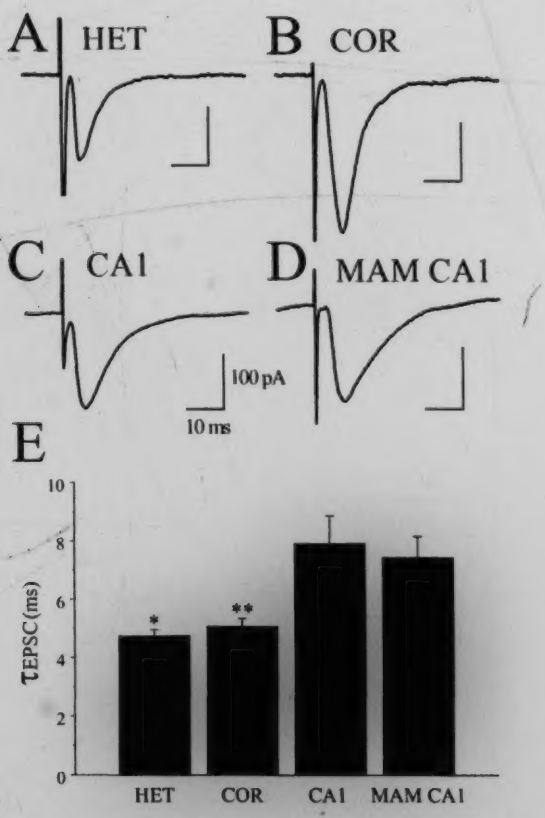

Figure 2-4. Time constants of EPSCs in each cell type. *A-D*: these traces show typical EPSC waveforms observed in each corresponding cell type at –75 mV. Note that the heterotopic and cortical EPSCs appear more rapid than those of normal or normotopic CA1 neurons. *E*: The time constants of decay of EPSCs (τ_{EPSC}) in untreated CA1 (CA1) and normotopic CA1 neurons in MAM-treated rats (MAM CA1) are much slower than those of EPSCs in layer 2-3 cortical (COR) and heterotopic (HET) pyramidal cells. * - statistical significance in comparison with CA1 neurons from untreated animals, * - $P<0.0001$; ** - $P<0.0016$.

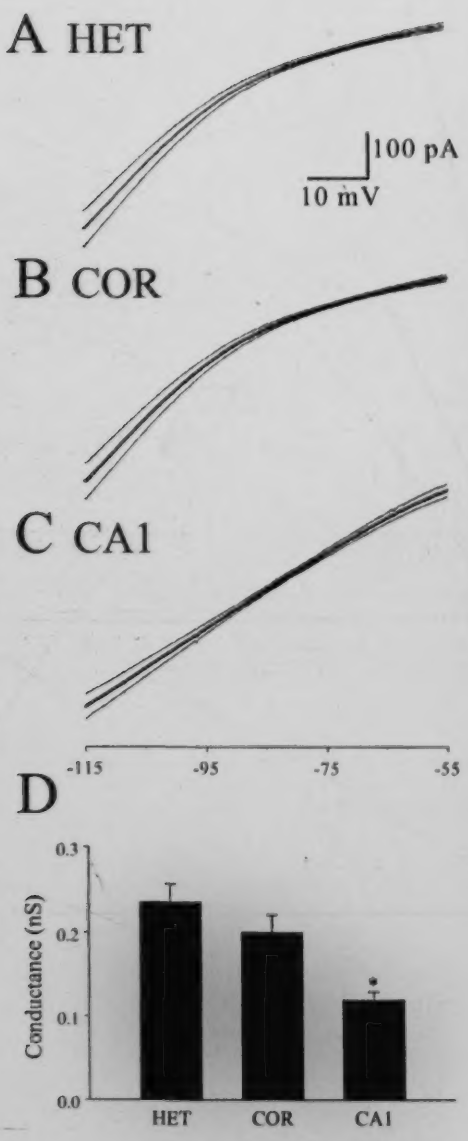

Figure 2-5. Comparison of the steady-state current-voltage relationship in heterotopic, and cortical and CA1 pyramidal cells from untreated animals. *A-C*: Traces of mean (± sem) membrane current elicited by a slow voltage ramp from –115 mV to –55 mV (n=13 for each). Note the sharp increase in conductance at more negative membrane potentials in heterotopic and layer 2-3 cortical neurons compared with CA1 pyramidal cells. *D*: Comparison of chord steady-state conductance measured between –115 mV and –100 mV in the cell types above. Membrane conductance in heterotopic and cortical neurons was significantly greater than in CA1 neurons between these potentials. * - statistical significance in comparison with cortical neurons. * - $P<0.0019$.

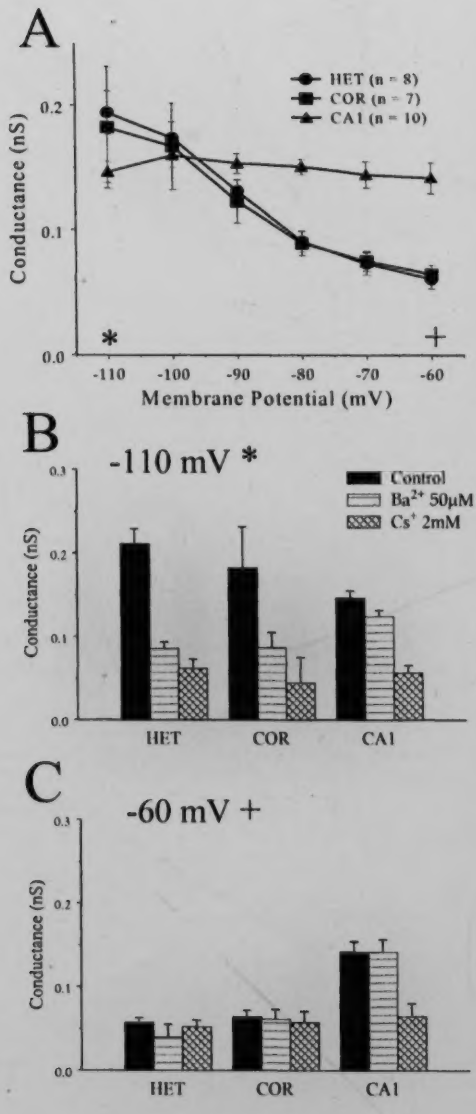

Figure 2-6. Comparison of Ba^{2+} and Cs^{+} effects on steady-state membrane conductance in heterotopic, cortical, and CA1 pyramidal cells. *A*: Plot of membrane conductance vs. potential in saline, for each cell type. Note that CA1 neurons display a fairly linear level of conductance across all membrane potentials, compared with the increased conductance at more negative potentials in heterotopic and layer 2-3 cortical neurons. *B* and *C*: Ba^{2+} and Cs^{+} both strongly inhibit membrane steady-state conductance between –115 and –105mV in heterotopic and cortical neurons, while having no significant action between –65 and –55mV. Cesium inhibits membrane conductance at all potentials in CA1 pyramidal cells, but barium only has a weak effect between –115 and –105mV.

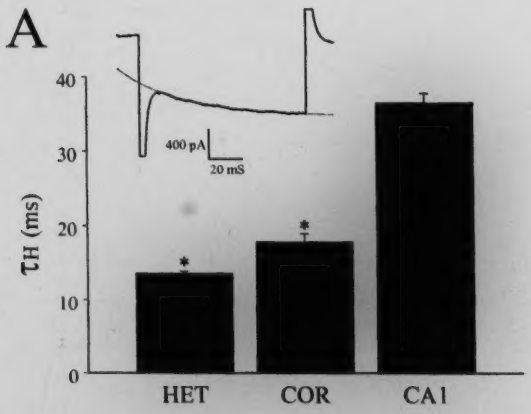

Figure 2-7. The time constants (τ_H) of membrane current elicited by a voltage step from -75mV to –115mV. *A*: The time constant of current decay over 200 ms at –115 mV is much slower in CA1 neurons than in either heterotopic or layer 2-3 pyramidal cells. *Inset*: An example of the exponential fit used to determine the τ_H values in *A*. * - statistical significance in comparison with CA1 neurons from untreated animals. * - $P<0.0001$.

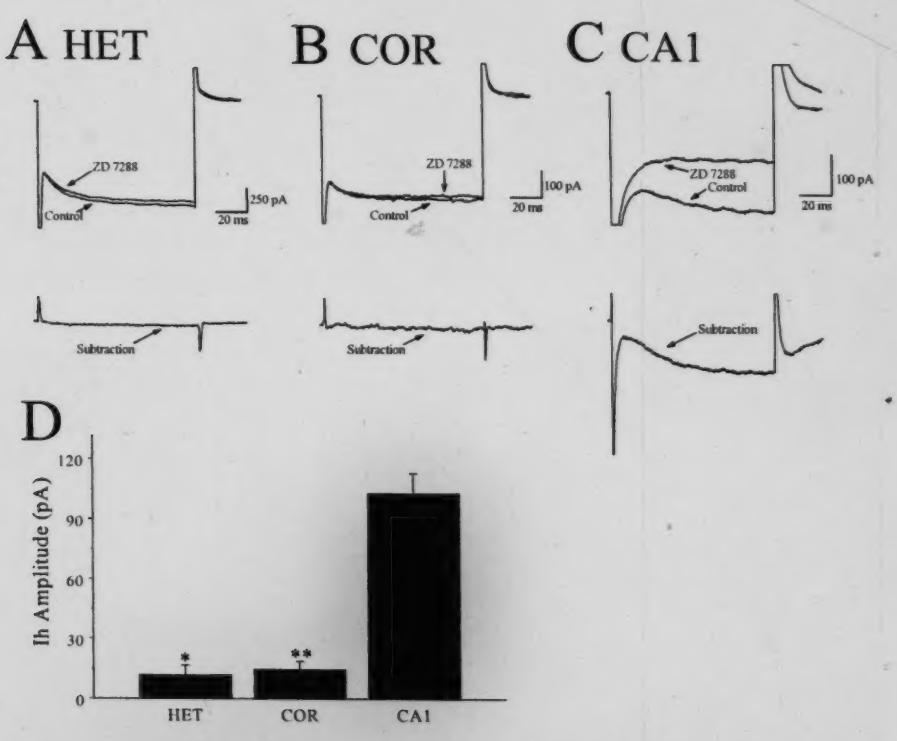

Figure 2-8. CA1 neurons display a prominent I_h, but layer 2-3 cortical and heterotopic neurons do not. *A-C*: The effect of ZD 7288, the I_h blocker, on current elicited by a voltage step to –115 mV from –75mV. Current traced in control and in the presence of 50 μM ZD 7388 are shown superimposed. The subtracted traces below show the isolated I_h in each type of cell. *D*: the peak amplitude of I_h in all three cell types. * - statistical significance in comparison with CA1 neurons from untreated animals. * - $P<0.0008$; ** - $P<0.0044$.

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Chapter III: Conclusions

With these experiments we have become the first to demonstrate an altered effect of NPY in the MAM model of cortical dysplasia and epilepsy, and have added substantially to the growing collection of evidence that ectopic neurons in the hippocampi of these animals were originally fated to reside in the neocortex. However, with these results come further questions as to the workings and pathogenesis of the epileptic, dysplastic brain. For example, how do these cortical neurons end up in the hippocampus, and why do the intraheterotopic inputs lack NPY receptors? Perhaps more importantly, how applicable is the MAM model to the human condition of focal cortical dysplasia? These issues, and others, will be addressed in this section, along with data not discussed in the previous chapter.

Our results show that NPY has only a very weak effect on the EPSCs of heterotopic neurons when the stimulating electrode is placed within the hippocampal heterotopias of MAM rats. Because NPY acts presynaptically (Colmers et al., 1987, 1988), this suggests that the excitatory inputs to heterotopic neurons have few, if any, NPY receptors on their terminals. The origin of excitatory inputs within the heterotopia is unclear, but evidence suggests strongly that the en passant fibres of the stratum radiatum are not an exclusive source. Earlier studies using carbocyanine tracing indicate that stratum radiatum fibers avoid neurons of the heterotopia (Chevassus-au-Louis et al., 1999a), consistent with their expressing a complement of cell surface markers suppressing their integration within the hippocampus. This raises the

possibility that a considerable number of excitatory inputs originate within the heterotopia itself. As the intra-heterotopic inputs appear to lack NPY receptors, heterotopic-heterotopic excitation may contribute to epileptiform activity found in these ectopic neurons (Baraban et al., 2000), and may also be a factor in human neuronal migration disorders where dysplastic areas frequently form epileptic foci (Palmini et al., 1991). However, the reason why heterotopic neurons in area CA1 of the hippocampi of MAM rats do not express NPY receptors is not clear. One possibility is that the lack of NPY innervation to these cells has had an effect on the NPY receptors, as immunohistochemical staining for the peptide itself shows that the heterotopia does not contain NPY, while the surrounding SR fibers do (SC Baraban, personal communication). Because the SR fibers avoid the heterotopia (Chevassus-au-Louis et al., 1999a), it is possible that a lack of NPY release within the heterotopia may lead to downregulation of its receptors by the ectopic neurons. Another reason that these cells may lack NPY receptors is because they were, presumably, originally fated to reside in the neocortex. In our experiments, we found that NPY had a similarly weak inhibitory effect on the amplitude of normal layer 2-3 cortical neurons' EPSCs, with the stimulating electrode placed in layer 1 (14.01 ± 3.309%, n=7), as it did on intra-heterotopic connections in MAM rats. Additionally, immunohistochemical staining studies have shown that there are relatively few NPY receptors (Migita et al. 2001), and lower levels of NPY mRNA (Hendry, 1993) localized to layer 2-3 of the neocortex. As neuronal phenotype is largely specified during the final mitotic division within the ventricular zone (McConnel and Kaznowski, 1991), the apparent lack of NPY receptors on heterotopic neurons in

MAM rats may be due to the fact that these displaced layer 2-3 pyramidal cells normally do not express the receptor proteins.

We have shown that the NMDA receptor antagonist APV does not have as strong an inhibitory effect on the EPSCs of heterotopic neurons as it does on those of layer 2-3 cortical and CA1 pyramidal cells. This reduced effect of D-APV in hippocampal heterotopic neurons agrees with studies showing a relatively minor NMDA component in the EPSCs of ectopic neurons in the freeze-lesion model of cortical dysplasia (Luhmann and Raabe, 1996; DeFazio and Hablitz, 2000). Appropriately, molecular research shows that NMDA receptor mRNA is expressed at lower levels in MAM heterotopias than in the neocortex or hippocampi of control animals (Rafiki et al., 1998). While this reduced NMDA effect may seem contradictory to the hyperexcitable nature of these neurons (Baraban et al. 2000), an AMPA-receptor mediated hyperexcitability has also been reported in a chronic model of temporal lobe epilepsy (Lothman et al., 1995). As NMDA is known to play a role in learning and memory (Castellano et al., 2001), functions mediated primarily by the hippocampus (Sarvey et al., 1989), the poor memory seen in MAM rats (Shimizu et al., 1991) may be due, in part, to the reduced NMDA effect in hippocampal heterotopias.

In our study we found that, while hippocampal heterotopic neurons of MAM rats had relatively few physiological properties in common with normotopic or normal CA1 neurons, most properties studied were indistinguishable from those of layer 2-3 pyramidal cells. Similarities between heterotopic neurons and layer 2-3 pyramidal

cells included the shape of their EPSCs, a resting membrane potential of –75 mV, an inwardly rectifying potassium current (I_{IR}), and the absence of an inwardly rectifying cation current (I_h). These data, in combination with previous studies that found molecular similarities between these cell types (Chevassus-Au-Louis et al., 1998a,b; Castro et al., 2002), suggest that heterotopic neurons in area CA1 of the hippocampi of MAM rats were originally fated to reside in the neocortex. However, it is not known exactly how these neurons come to rest in the hippocampus. In addition to the hippocampal heterotopias, injection of MAM into pregnant rats on gestational day 15 causes the neocortex to be reduced in thickness, with only layers 5 and 6 intact (Jones et al., 1982). As well, it has been shown that the injection of MAM on this date also causes diffuse structural abnormalities in radial glial cells (Zhang et al., 1995), which normally provide pathways for young migrating neurons to their final destinations (Sidman and Rakic, 1973; O'Rourke et al., 1992). However, the glial scaffolding in the hippocampus is not affected by MAM injection on gestational day 15 (Zhang et al., 1995). It may be hypothesized, then, that parent cells for layer 2-4 neocortical neurons that avoid destruction by MAM develop into neurons that must migrate without the aid of radial glia. These neurons may aberrantly be attracted to the radial fibers of the hippocampus and proceed to form heterotopias in area CA1, creating synaptic connections with both the hippocampus and with the neocortex, the network to which they were originally committed (Chevassus-au-Louis et al., 1998b). This functional "bridge" between the hippocampus and neocortex (Chevassus-au-Louis et al., 1998b; Colacitti et al., 1998), as well as the hyperexcitability of the ectopic neurons (Baraban et al., 2000), contribute to the reputation of the MAM model as a an

important tool through which the mechanisms of epileptogenesis associated with dysplastic tissue may be studied. However, the question as to the relevance of the MAM model to the human condition has not been sufficiently addressed.

Epilepsy is the most common manifestation of neuronal migration disorders (Aicardi, 1994), and patients with cortical dysplasia suffer from seizures that are often medically intractable and may be life-threatening (Desbiens et al.,1993). One of the major problems with the MAM model of cortical dysplasia and epilepsy is the fact that spontaneous seizures have never been observed in MAM rats. Moreover, of all the animal models of cortical malformation available, only the genetic models display spontaneous seizure activity (Chevassus-au-Louis et al., 1999b). However, isolated heterotopic tissue from MAM rats has been shown to support epileptiform burst generation independent of synaptic input, while isolated dysplastic human tissue has not (Baraban et al., 2000), and continuous video-EEG monitoring of epileptic activity, the only experiment that would absolutely rule out the possibility of spontaneous seizures in MAM rats, has never been reported (Chevassus-au-Louis et al., 1999b). As well, many cases of cortical dysplasia in humans presumably go undiagnosed, due to lack of symptoms (i.e. seizure activity).

Another slight problem with the MAM model is that heterotopias constitute only a small percentage of the neocortical malformations that may lead to epilepsy in humans. Although microscopic neuronal heterotopias were observed in nearly half of those in a large group of patients with NMD, they were usually accompanied by more

diffuse abnormalities and none were observed within the hippocampus (Mischel et al., 1995). However, the hippocampal heterotopias of MAM rats are morphologically similar to heterotopic nodules found in human periventricular or subcortical nodular heterotopia (PNH) (Colacitti et al., 1998) in which multiple coalescent nodules are located at the border of the lateral ventricles, extending along the temporal horns of the ventricles to reach the hippocampal formation (Battaglia et al., 1996, 1997). Nodular heterotopias, either subcortical or periventricular, are the most common and least severe form of NMD (Dubeau et al., 1996). As in the MAM rats, patients with this condition also exhibit thinning of the neocortex (Dubeau et al., 1995), and neurons present at the borders of the PNH nodules (Battaglia et al., 1996) are similar to marginal fusiform neurons found in MAM animals (Colacitti et al., 1998). Especially relevant to our study is the finding that periventricular nodules are largely made up of displaced neocortical neurons (Dubeau et al., 1995), as are evidently the heterotopias of MAM rats. In addition, reciprocal connections made by the heterotopias to the neocortex in MAM rats (Chevassus-au-Louis et al., 1998a, b) have also been suggested in human PNH by the presence of fibers radiating from the nodules (Spreafico et al., 1998). Therefore, the abnormal synapses that form a functional connection between the hippocampus and neocortex in MAM rats (Chevassus-au-Louis et al., 1998b) may help explain the propagation of abnormal neuronal discharges and epileptiform activity to the neocortex, hippocampus, and periventricular nodule in patients with PNH (Dubeau et al., 1995). Finally, MAM animals seem to share the same pharmacoresistant profile that is common in patients with PNH (Smyth et al., 2002). Although MAM rats may not provide the best model

for generalized NMDs, its similarities with human PNH make it a useful tool for studying this type of brain dysgenesis.

Interestingly, PNH in humans is much more common in females, suggesting sex linked autosomal dominant inheritance (Dubeau et al., 1995). Other NMDs, such as types I and II lissencephaly, have also been shown to be caused by genetic factors (Palmini et al., 1994). However, the vast majority of cortical dysplasias occur sporadically with no detectable cause. Some reports in the literature support the etiologic role of specific prenatal harmful exposures in the genesis of different NMDs in humans, such as methylmercury intoxication (Choi et al., 1978), cytomegalovirus infection (Norman et al., 1976), ionizing radiation (Rakic, 1988), alchohol (Peiffer et al., 1979), and isotretinoin (Volpe, 1987). Prenatal physical trauma has also been shown to cause cortical dysplasia in some cases, as well as exposure to over-the-counter medication during the first half of gestation (Palmini et al., 1994). With so many different causes of NMDs, known and unknown, it is possible that exposure to a DNA-methylating agent such as MAM during the early stages of gestation may lead to PNH or other types of heterotopias in the offspring. For example, smokers may put their children at risk because nitrosamines found in tobacco products readily methylate DNA (Hecht, 1999). In fact, pregnant women who inhale second-hand tobacco smoke containing teratogenic and carcinogenic compounds, which are reportedly not detoxified in the placenta, have been shown to be at higher risk for malformations of the fetus (Remmer, 1987). As nitrosamines are also found in many common foods such as garlic (Milner, 2001) and beer (Lijinsky, 1999), prenatal

exposure to such DNA-methylating agents may go unnoticed by the mother and may not be considered as causative if cortical dysplasia arises in the offspring. For this reason, data collected from the MAM model may be especially relevant to the human condition, even if the physical effects of MAM do not precisely replicate those of human DNA-methylating teratogens.

The redirecting of neocortical neurons to the hippocampus in MAM rats, and the diffuse abnormalities in the brains of patients with NMDs, make it clear that any sort of insult to a developing embryo can drastically alter the phenotype of the offspring. The migration of neurons to their final destinations in the CNS is certainly a delicate process, and its interruption may lead to devastating results. One of the most important things learned from the MAM model is this seemingly obvious conclusion. In an industrial society such as ours, exposure to carcinogens and dangerous chemicals is a daily occurrence. Clearly, teratogens such as MAM have an important role to play in the study of the toxicity of environmental toxins and medications to pregnant women and their fetuses.

Neuronal migration abnormalities, once a pathological curiosity, have come to occupy a first place rank among neurological disorders of childhood (Aicardi, 1994). However, "malformations of every conceivable kind, degree, and combination occur, and no two of them are ever alike" (Willis, 1962). With such a diverse range of irregularities that may be caused by NMDs, the MAM model is an important tool for

studying the pathology of some, but not all, types of cortical dysplasia. The relation of the MAM model to PNH, and its possible connection to human teratogens make MAM rats especially useful for the study of the etiology of NMDs and the epileptogenicity of malformations such as periventricular nodules. The finding that NPY has only a very weak effect within MAM heterotopias may be particularly relevant to the latter. As NPY is known to play an important role in seizure modulation (Vezzani et al., 1999), a deficiency of this inhibitory peptide in cortical dysplasias, particularly periventricular nodules, may contribute to hyperexcitability. If this is the case, as only further studies on human subjects will show, it may help explain the correlation between NMDs and epilepsy, and may lead to a novel anticonvulsant therapy for these pharmacoresistant disorders.

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